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Novel approach to prepare biopolymers based nanocrystals

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ABSTRACT

New techniques developed to prepare biopolymer nanocrystals from easily available starch and protein source. Though nanocrystals preparation has been reported by acid hydrolysis in case of starch nanocrystals, some adverse effects may be noted when used as fillers. Starch crystals are prepared by addition of plasticizer further followed by homogenization and ethanol wash followed by freeze drying. Particle size of pea starch observed by Transmission Electron microscopy is 50-100nm and the morphology is determined by Scanning electron microscopy. Certain repetitive solubilization and crystallization method has been tried out in case of protein nanocrystals. Results from Scanning electron microscopy and Transmission Electron microscopy can be noted that the particle size of first stage protein isolate is of 870nm wheras after optimization of last stage (final solubilization and crystallization) results in 350nm. Thus there is a tendency of size reduction in case of protein crystals.

Keywords: pea, protein, starch, nanocrystals

INTRODUCTION

Nanocrystals of starch do have some outstanding properties. They are likely to be improving the films characteristics by enhancing the mechanical properties when incorporated into them. Preparation of nanocrystals from starch has been reported by the HCL acid hydrolysis (1), chemical modification of starch nanocrystals: has been reported by chemical reaction with small molecules(2), grafting onto polymer chains with coupling agents, and grafting from polymer chains with polymerization of a monomer(3). The conventional method of starch nanocrystals preparation is acid hydrolysis (4).

So far no reports of either edible protein nanocrystals preparation or modification have been cited. Thus the new technique of repetitive solubilization and crystallization process can lead to a new dimension of research. These protein nanocrystals can be used as fillers in edible laminates to enhance thermal and mechanical.

MATERIALS AND METHODS

2. Experimental Section:
Green pea seed, Green pea starch (extracted in laboratory), Glycerol, Polyethylene glycol (400), Ethanol (Merck, India) used as received. Double distilled (DD) water was used for preparation of cow pea starch and cow pea protein isolate.

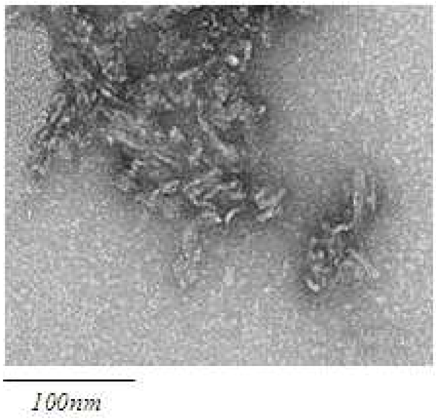
2.1. *Preparation of pea special protein Isolate:*
The dehulled meal from green pea was ground in a grinder and the resulting flour was sieved by sieving machine in 35 mesh. The pea flour was then treated with 0.1M NaOH (w/v) solution. The resulting mixture was stirred for an hour on a magnetic stirrer. Stirred mixture was centrifuged at 6000rpm for 15 minutes. The sediment was discarded and supernatant was taken and its pH was adjusted to 4.5 which was further vacuum dried at 40°C for 8 hours. Then this resolubilisation and crystallization process was carried out for at least 6 times.

2.2 *Preparation of starch nanocrystals:*
Green technology developed for Nanostarch crystals preparation comprises mainly two ways; a) Pea Starch added to polar solvents like glycerol, Polyethylene glycol 400 and gelatinized at 70°C for a certain period of time till gelatinization occurs. Further such gelatinized starch is treated with ethanol and nano crystals of starch are obtained. b) Starch first homogenized with Glycerol and later ethanol washed by centrifugation at 6000rpm for 15minutes and the residue is collected.

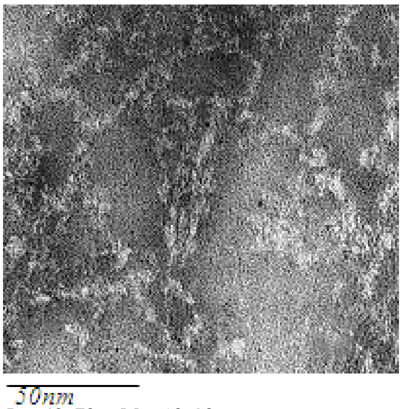
2.3. *Characterizations:*
The morphology of the rice starch films was observed with a Scanning Electron Microscope (SEM, HITACHI SEM (S-3400N, India) and TEM. The size of the pea starch and starch nanocrystals was observed using a differential light scattering technique by laser analyzer. (LS-230, Coulter, U.S.A.).

RESULTS AND DISCUSSION

(TEM of starch nanocrystals)



L = 60–150nmW = 15–30nm
Fig1a. Pea Starch



L = 40–70nmW = 10–20nm
Fig.2b. Pea starch nanocrystals

The results of TEM for starch crystals denotes that by the above mentioned technique, starch nanocrystals can be made ,which can be further used as fillers in control films to improve film characteristics. As reports of acid treated nanocrystals are observed, this type of green technology based nanocrystals of starch are not so far cited. Some nano porous structures are observed in starch films incorporated with acid treated nanocrystals (4) which creates void space thus may weaken the physical properties of the films. Though it may not form in case of such green technology based nanocrystals of starch.

Scanning Electron Microscopy

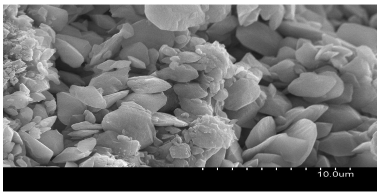


Fig2a. Single stage pea protein isolate
(c.a.870nm)

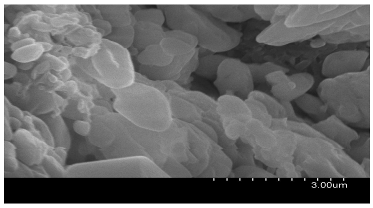


Fig2b.Multistage pea protein isolate
(c.a.400nm)

In fig. 2a and Fig.2b. Morphology of the protein isolate crystals of both special pea protein isolate (1st stage) and the special pea protein isolate after repeated crystallization steps are noted. The morphology denotes a globular arrangement which is of unique structure and dimension in the later case the dimension is reduced which may be resulted from repeated crystallization process. The particle size of the aggregate for the 1st stage protein isolate crystals is 390nm and the aggregate of the multi-stage protein crystals is of 330nm denoted by laser particle size analyzer.

CONCLUSION

The approach of making these types of starch and protein nanocrystals is expected to be a new one. These types of reports are not so far observed. Common technique of preparing starch nanocrystals is acid treatment which may lead to certain issues like weakening of the films and laminates due to void space formation by acid treatment creating nanopores in them. Thus to overcome such problem we can try to substitute such nanocrystals with green technology based nanocrystals of starch which is expected to improve the mechanical and other physical properties when incorporated into films. The multistep processed protein isolate preparation is also a new technique. The particle size analyzer reports that the protein aggregate ranges from 330nm -390nm, thus this technique can also be considered as a new approach of preparing protein at nanoscale. Thus biopolymer materials themselves can be used instead of synthetic nonmaterial as fillers in film matrices.

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